Running title: *Floracarus* ultrastructure

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An ultrastructural study of the relationship between the mite *Floracarus perrepae* Knihinicki & Boczek (Acariformes:Eriophyidae) and the fern *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae)

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ABSTRACT

The ultrastructure of *Floracarus perrepae* was investigated in relation to its host, *Lygodium microphyllum*.   Feeding by the mite induces a change in epidermal cell size, and cell division is stimulated by mite feeding, causing the leaf margin to curl over into a roll with two to three windings. The enlarged epidermal layer greatly increases its cytoplasmic contents, which become a nutritive tissue for the mite and its progeny. The structure and depth of stylet penetration by the mite, and the thickness of the epidermal cell wall of *L. microphyllum*, does not appear to account for the mite's differential ability to induce leaf rolling in its co-adapted host from southeast Queensland but not in the invasive genotype of the fern in Florida.

KEY WORDS

INTRODUCTION

Floracarus perrepae Knihinicki & Boczek (Acariformes:Eriophyidae) is a herbivore associated with *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae), the Old World climbing fern (Knihinicki & Boczek 2002; Goolsby *et al.* 2003a). *Lygodium microphyllum* is native to the Old World wet tropics and subtropics including Africa, Australasia, Asia, and Oceania (Pemberton 1998). *Lygodium microphyllum* is an aggressive invasive weed of moist habitats in southern Florida (Pemberton & Ferriter 1998), and the target of a biological control program (Pemberton *et al.* 2002, Goolsby *et al.* 2003a). *Floracarus perrepae* is one of the herbivores under consideration as a biological control agent. Several distinct genotypes of *F. perrepae* have been identified (Goolsby *et al.* 2003b). The genotype of the mite from Southeast Queensland performed poorly on the invasive form of *L. microphyllum* from Florida. The mechanism for the mite-fern interaction is not known. However, the ability of the mite genotype to feed and induce gall tissue and leaf rolling in the fern is critical to its ability to survive and reproduce. Mature females prefer the new sterile subpinnae of actively growing plant tips for oviposition. Observations of newly formed subpinnae leaf rolls revealed up to five adults inside, although a single gravid female is able to cause the development of the deformation (curled subpinna) for itself and all its progeny. As the subpinna margin continues to roll, the number of mites may increase significantly, so that subpinnae leaf rolls ultimately may contain 30 or

more adults, juveniles, and eggs (Ozman SK & Goolsby JA, 2002, unpublished data.)

Eriophyoid mites are small, with body lengths ranging from 80 to 500 μm and an average length of approximately 200 μm. These mites are highly specific, living and reproducing only on susceptible host-plant species (Westphal & Manson 1996). Mouth parts of eriophyoid mites are complex and are uniquely adapted for obligative phytophagous feeding associated with host plants (Nuzzaci 1979a, Thomsen 1987, Lindquist & Oldfield 1996). The eriophyoid mite gnathosoma includes short pedipalps, a prominent subcapitulum (rostrum), and the stylets. Depending on interpretation, stylet number ranges from seven to nine (Lindquist 1996, Nuzzaci 1979b). Both Lindquist (1996) and Nuzzaci and Alberti (1996) have identified nine stylets including the unpaired oral stylet; the inner infracapitular (auxiliary) stylets; the outer infracapitular stylets; and the cheliceral stylets, which divide into fixed and movable digits. The oral stylet is only about half the length of the cheliceral stylets. Movement of the cheliceral stylets is limited to an alternate back-and-forth boring motion activated by the motivator, and may continue throughout the feeding process (Lindquist & Oldfield 1996).

According to Thomsen (1988), enzymatic digestion of the epidermal wall precedes food ingestion. Westphal and Manson (1996) reported that it is unlikely that salivary deposition on the host surface is involved in enzymatic

dissolution of the cell walls prior to insertion of the cheliceral stylets. Lindquist (1996) and Nuzzaci and Alberti (1996) have provided an excellent review of the literature describing the structure and function of eriophyoid mites.

Penetration of the mouth parts is generally restricted to the epidermal layer of the host plant (Oldfield 1996), and feeding activity results in minimal physical and physiological damage according to Lindquist and Oldfield (1996). The cheliceral stylets of gall mites were found to penetrate only about 2 μm, and affected only the host cell wall (Westphal & Manson 1996). Prior to penetration, the pedipalps are positioned against the host-plant surface, with the terminal palpal segments anchoring the gnathosoma (Nuzzaci & Alberti 1996).

This study was designed to describe both the morphology of *F. perrepae*, especially the mouthparts, and the ultrastructural changes in *L. microphyllum* subpinnae (smallest leaf units) as a result of mite feeding. Detailed knowledge of the morphology of the mouthparts of *F. perrepae* is essential to the understanding of its feeding behaviour.

MATERIALS AND METHODS

The *F. perrepae* used in the study were collected from *L. microphyllum* at Carbrook Creek, Logan, Queensland; and Scrub Hen Creek, Lockhart River, Queensland by John A. Goolsby. Infested subpinnae with tightly rolled margins

were removed from the compound pinnae and prepared for light and electron

microscopy. Some subpinnae were fixed and dehydrated in acidified DMP (2,2-

dimethoxypropane). Other infested subpinnae were dropped into boiling water

for 30 seconds, then fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4).

 Following fixation, both samples were dehydrated in a graded ethanol series

and then critical-point dried using liquid carbon dioxide as a transitional fluid.

Specimens were mounted on aluminum stubs using double sticky carbon tape,

sputter coated with gold or gold-palladium, and examined using a JEOL JSM

6400 or 6300 scanning electron microscope. Infested pinnae were prepared for

light and transmission electron microscopy by fixation in 2.5% phosphate-

buffered glutaraldehyde (pH 7.4), post-fixed in buffered osmium tetroxide (pH

7.4), dehydrated in a graded acetone series, embedded in epon-araldite epoxy

resin, and ultrathin sectioned. For transmission electron microscopy, the tissue

was stained *en bloc* with uranyl acetate, post-stained with lead citrate, and then

examined on a JEOL 100CX microscope. For light microscopy, resin sections

were cut (2-5 μm thick), stained with toluidine blue, and examined using an

Olympus BH-2 microscope.

RESULTS

 Mites removed from *L. microphyllum* subpinnae are shown in Figs. 1 and

2. The general morphology is of the vermiform type, characterized by a

complex gnathosoma, prodorsum, opisthosoma, and two pairs of legs. The

opisthosoma has three pairs of ventral setae and numerous rows of small

annuli (Figs. 1-3). Accessory setae are absent from the opisthosoma (Fig. 3).

The ends of the legs have featherclaws and solenidia (Figs. 4, 5). Unguinal

setae are absent from the tarsi. The genital region is on the ventral surface,

just posterior to the legs (Fig. 2).

Mouthparts of these eriophyoid mites are complex and highly adapted for

feeding on the host plants. The gnathosoma includes short pedipalps, a

prominent subcapitulum (rostrum), and the stylets. During feeding, the

pedipalps (Figs. 6, 7) form a suction type of attachment to the host plant while

the anal sucker anchors the posterior end (Fig. 8). Damage to the fern

epidermal wall, caused by stylet probes at feeding sites, was evident throughout

the pinnae curls (Fig. 9). The stylets are visible only when the mite is feeding.

There appear to be nine stylets in the stylet sheath of the mite. The most

prominent stylets are the paired cheliceral stylets. These stylets taper toward

their apex, and each is divided into the digitus fixus, a fixed digit; and the digitus

mobilis, a movable digit (Figs. 6, 10, 11). The cheliceral stylets are the first to

penetrate the host pinna epidermal cells. The two pedipalpal stylet pairs are

known as the inner infracapitular (auxiliary) stylets and the outer infracapitular

stylets (Figs. 10, 12). The bases of two pairs of pedipalpal stylets are marked in

Figure 10. The distal ends of the pedipalpal stylets are shown in Figure 12.

The central unpaired stylet is the oral stylet (labrum) (Figs. 10, 12).

The mesophyll layer of the *L. microphyllum* subpinna is composed of relatively uniform parenchyma cells (Fig. 13) and scattered vascular bundles. The epidermal layers have few chloroplasts and limited cytoplasm (Fig. 14). Mite feeding on the subpinna epidermis induces rolling, and on some subpinnae the whole margin may be affected. Increased epidermal cell size and cell division is stimulated by mite feeding, and results in the leaf margin rolling over, either upward or downward, and inward (Fig. 15) on itself as many as two or three times. An epidermal cell-wall extension develops in response to *F. perrepae* feeding activity (Fig. 16). Mite feeding also stimulates the epidermal layer to become a nutritive tissue (Figs. 17, 18). The enlarged epidermal cells greatly increase their cytoplasmic contents as the subpinna continues to roll (Figs. 17-19). Continued feeding by the adults and immatures leads to subpinna necrosis (Fig. 20) and premature defoliation of *L. microphyllum*, gradually debilitating the plant over time. The subpinna eventually dries and falls off the frond, before which time the adults move to another young subpinna to feed and initiate new curls. Eventually, the mite-induced leaf necrosis and death equals the level of new growth that appears.

We found that the *F. perrepae* genotype collected from Southeast Queensland (Logan) was not able to induce leaf rolling in the invasive *L. microphyllum* genotype from Florida, but it readily induced leaf rolls in the local

Southeast Queensland genotype of the fern. Differences in the structure of the epidermal layer of *L. microphyllum* from the Southeast Queensland and Florida genotypes were investigated to determine if these could account for the differences in acceptance by *F. perrepae*. We were unable to identify any specific morphological or anatomical differences between the ferns from different locations that might account for the different level of acceptance by the mite.

DISCUSSION

Knihinicki and Boczek (2002) described this mite and mentioned that there are no unguinal and caudal setae, as confirmed in this study.

Lindquist (1996) and Nuzzaci and Alberti (1996) stated that Eriophyidae have seven stylets, while some taxa, particularly Phytopidae and Diptilomiopidae, have nine stylets. *Floracarus perrepae* belongs to the family Eriophyidae, but the evidence of this study points to the existence of nine stylets because there appear to be two pairs of pedipalpal stylets.

The feeding mechanism of *F. perrepae* is poorly understood, and further interpretation of the function of the various components of the gnathosoma is required. Feeding by *F. perrepae* on *L. microphyllum* stimulates the development of nutritive tissue, causing the formation of tight leaf rolls on the subpinnae; the leaf rolls maintain an environment favorable for the mite. The

substantial difference in vulnerability of the two genotypes of *L. microphyllum* to the feeding activity of *F. perrepae* does not appear to be related to mite stylet length or plant epidermal cell wall structure. A biochemical basis for the successful interaction of the adapted genotype of the mite and fern should be investigated. In addition, the best-adapted genotype of the mite for use as a biological control agent should be selected based on its ability to induce large leaf rolls with nutritive tissue. Our findings illustrate the complex and profound interactions of eriophyid mites and their hosts. As for *F. perrepae*, this interaction with the two forms of *L. microphyllum* emphasizes its extremely narrow host specificity and potential to impact its host. This function of the biology of *F. perrepae* is the basis for its efficacy and safety as a biological control agent.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Fig. 1. Dorsal view of *Floracarus perrepae*.

Fig. 2. Ventral view of *Floracarus perrepae* female with genital aperture (arrow).

Fig. 3. Posterior view of opisthosoma showing caudal seta (cs), third ventral seta (vs), and anal lobe (al).

Fig. 4. Dorsal view of anterior of the mite showing the gnathosoma and paired legs.

Fig. 5. Lateral view of tarsi I: empodial featherclaws (fc) and solenidia (s).

Fig. 6. Gnathosoma with cheliceral stylets (digitus fixus (df) and digitus mobilis (dm)) extended, and cheliceral retainers (cr), basal (palpcoxal) setae (bs), and pedipalp (p).

Fig. 7. Distal ends of palps forming "suction pad".

Fig. 8. *Floracarus perrepae* on the abaxial surface of a *Lygodium microphyllum* subpinna.

Fig. 9. *Floracarus perrepae* feeding sites on the epidermal surface of *Lygodium microphyllum*.

Fig. 10. Complete set of *Floracarus perrepae* stylets: digitus fixus (df), digitus mobilis (dm), oral stylet (os), pedipalpal stylets (ps), cheliceral retainer (cr), and basal seta (bs).

Fig. 11. Lateral view of the cheliceral stylets: digitus fixus (df) and digitus

mobilis (dm).

Fig. 12. Oral stylet (os) and pedipalpal stylets (ps) of *Floracarus perrepae*.

Fig. 13. Scanning electron micrograph of surface and sectional view of a non-curled area of a *Lygodium microphyllum* subpinna.

Fig. 14. Transmission electron micrograph of *Lygodium microphyllum* abaxial epidermis in a non-rolled area of the subpinna.

Fig. 15. Light micrograph of a subpinna curl showing enlarged nutritive abaxial epidermis.

Fig. 16. Transmission electron micrograph of an epidermal cell-wall extension (arrow) developed in response to *Floracarus perrepae* feeding activity.

Fig. 17. Transmission electron micrograph of the early stage of nutritive epidermal cell development within the subpinna leaf roll.

Fig. 18. Scanning electron micrograph of a surface and cross-sectional view of enlarged nutritive epidermal cells (arrows).

Fig. 19. Transmission electron micrograph of nutritive tissue in the subpinna curl.

Fig. 20.   Transmission electron micrograph of necrotic epidermal and mesophyll tissue caused by continued *Floracarus perrepae* feeding activity.